



# Bioenergy cropping systems that incorporate native grasses stimulate growth of plant-associated soil microbes in the absence of nitrogen fertilization



Lawrence G. Oates<sup>a,b,\*</sup>, David S. Duncan<sup>a,b</sup>, Gregg R. Sanford<sup>a,b</sup>, Chao Liang<sup>a,c</sup>, Randall D. Jackson<sup>a,b</sup>

<sup>a</sup> DOE-Great Lakes Bioenergy Research Center, University of Wisconsin-Madison, Madison, WI 53726, USA

<sup>b</sup> Department of Agronomy, University of Wisconsin-Madison, Madison, WI 53706, USA

<sup>c</sup> Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, China

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## ABSTRACT

The choice of crops and their management can strongly influence soil microbial communities and their processes. We used lipid biomarker profiling to characterize how soil microbial composition of five potential bioenergy cropping systems diverged from a common baseline five years after they were established. The cropping systems we studied included an annual system (continuous no-till corn) and four perennial crops (switchgrass, miscanthus, hybrid poplar, and restored prairie). Partial- and no-stover removal were compared for the corn system, while N-additions were compared to unfertilized plots for the perennial cropping systems. Arbuscular mycorrhizal fungi (AMF) and Gram-negative biomass was higher in unfertilized perennial grass systems, especially in switchgrass and prairie. Gram-positive bacterial biomass decreased in all systems relative to baseline values in surface soils (0–10 cm), but not subsurface soils (10–25 cm). Overall microbial composition was similar between the two soil depths. Our findings demonstrate the capacity of unfertilized perennial cropping systems to recreate microbial composition found in undisturbed soil environments and indicate how strongly agroecosystem management decisions such as N addition and plant community composition can influence soil microbial assemblages.

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## 1. Introduction

The development of biofuels derived from cellulosic materials may catalyze an exceptionally rapid and extensive change in land use and land cover (Dale et al., 2011; Perlack et al., 2005) by dramatically expanding the range of cropping systems able to supply biomass feedstock. These systems include low-input perennial systems, which could provide substantial environmental benefits (Robertson et al., 2008). Many of these systems have received little attention in an agronomic context, and thus there is limited information about the ecological effects of managing them for biomass production (Dale et al., 2011; Ragauskas et al., 2006). One such effect of harvesting these novel cropping systems may be altered soil microbial abundance and composition.

The interactions of plants and microbes are major determinants of ecosystem function (van der Heijden et al., 2008). These interactions are particularly noticeable in plant-associated microbes such as mycorrhizal fungi and endophytes (Bonfante and Anca, 2009). Plants vary greatly in the degree to which they form these associations and in the taxonomic breadth of their potential partners (Hartmann et al., 2009; Philippot et al., 2013). Even in the absence of specific interactions, plants are the predominant source of reduced carbon (C) to soil systems (De Deyn et al., 2008; Philippot et al., 2013), so microbial biomass and composition are affected by the investment of plant resources belowground (Kätterer et al., 2011), as well as by the quantity and quality of their litter (Cleveland et al., 2014). Moreover, plant-microbe interactions are influenced by increased nutrient availability, where exogenous nutrient addition to the system, usually as nitrogen (N) and phosphorus (P), is typically detrimental to plant-associated and oligotrophic microbes (Leff et al., 2015). Thus, cropping system management activities such as manipulation of plant species composition, nutrient inputs, and biomass harvest

\* Corresponding author at: DOE-Great Lakes Bioenergy Research Center, University of Wisconsin-Madison, Madison, WI 53726, USA.  
E-mail address: [oates@wisc.edu](mailto:oates@wisc.edu) (L.G. Oates).

can significantly influence soil microbial composition and the ecosystem processes they drive.

We investigated biomass cropping system effects on soil microbial composition building on previous studies that (1) documented cropping system legacies in soil microbial composition (Liang et al., 2012) and (2) identified relatively rapid divergence (i.e., 2 years post-establishment) between corn and prairie cropping systems (Herzberger et al., 2014). Using lipid biomarkers we compared total and functional group microbial biomass and composition among the post-establishment cropping system  $\times$  management combinations at two soil depths. While interpreting biomarkers for phylogenetic resolution is less than desirable, and using biomarkers to elucidate diversity is flawed, lipid biomarkers are excellent for assaying microbial community composition and biomass, and are considered an excellent indicator of the fungal and bacterial components when comparing treatments (Frostegård et al., 2011).

## 2. Methods

### 2.1. Site description and management

The experimental site was located in south central Wisconsin, USA at the University of Wisconsin's Arlington Agricultural Research Station (AARS) in Arlington, WI (43°17'45"N, 89°22'48"W and 315 masl). Mean annual air temperature and precipitation are 6.9°C and 869 mm respectively (1981–2010, National Climate Data Center). For our study years, 2008 and 2013, average growing season (1 April through 31 October) air temperature was 14.5°C and 14.6°C, respectively. Growing season precipitation was 795 mm in 2008 and 736 mm in 2013 with month of soil sampling (August) precipitation being 45 mm and 42 mm in 2008 and 2013, respectively. Cumulative growing degree days were similar for both years (1286 in 2008 and 1330 in 2013) (Sanford et al., 2016). Soils at the site are classified as Plano silt-loam (fine-silty, mixed, superactive, mesic Typic Argiudolls with soil C > 20 g kg<sup>-1</sup>, N  $\geq$  0.19 g kg<sup>-1</sup>) developed over glacial till (Jokela et al., 2011).

To account for heterogeneity in the field arising from land use and land cover history, geologic, soil, aspect, or slope variation, the experiment was established using a fully-balanced randomized complete block design (RCBD) with five replicate blocks. Hence, all treatments were represented across land use histories of the recent past. Cropping system treatments represented in each block were continuous no-till corn (*Zea mays* L.), switchgrass (*Panicum virgatum* L.), miscanthus (*Miscanthus x giganteus* Greef & Deuter ex Hodkinson & Renvoize), hybrid poplar (*Populus nigra*  $\times$  *P. maximowiczii* A. Henry 'NM6') on a 6-year coppicing rotation, and a restored prairie, which is a mixture of 18 native prairie species (full crop and management details are given in Table S1). Prior to establishment of the experiment in May 2008, 2 of the blocks

consisted of corn (blocks 4 and 5) and 3 of the blocks were a mix of the cool-season grass timothy (*Phleum pratense* L.) and alfalfa (*Medicago sativa* L., blocks 1–3). In April 2008, all blocks were tilled and disked and the various treatments were established by May 2008. In June 2008, in the ~10-m buffers among the treatments and in several "blank" treatment plots that had been randomized but not assigned to a treatment, we sowed the cool-season grass  $\times$  *Festulolium* Asch. & Graebn. (var. Spring Green).

Each treatment plot within a block was 27  $\times$  43 m (0.12 ha) and was divided into main (17  $\times$  43 m) and sub-plots (10  $\times$  43 m). Nitrogen application rates to the corn main plot and corn sub-plots was based on spring soil tests and averaged 167 kg N ha<sup>-1</sup> y<sup>-1</sup> (5–14–42 granular starter fertilizer and 28–0–0 urea-ammonium nitrate side dress) over the study period. To provide a competitive advantage over weeds during establishment, N application (56 kg N ha<sup>-1</sup> of 34–0–0 granular ammonium nitrate) was delayed until early summer 2010 for the switchgrass and miscanthus main plots and the prairie sub-plot. This rate of N application continued on a yearly basis for the remainder of the study period. The poplar main plot received a single N application in 2010 of 210 kg N ha<sup>-1</sup> (34–0–0 granular ammonium nitrate). The sub-plots represented a departure from standard management practices and were (1) 100% stover residue retention for corn, (2) no N applied for switchgrass, miscanthus, and poplar, and (3) N applied for prairie.

### 2.2. Sampling and extraction

In August 2008, five 37-mm diameter soil cores were removed from the surface 25 cm of two "blank" plots from each of the 5 blocks (seeded to  $\times$  *Festulolium* in June 2008). The 5 subsamples from a plot (10 per block  $\times$  5 blocks = 50 samples) were extracted for microbial assay to represent the "baseline" microbial community. In August 2013, the same procedure occurred for each treatment plot within each block, except the 5 sub-samples within a plot were composited before extraction. At both time points, soils were split into two depths (0–10 cm and 10–25 cm) and placed in labeled plastic bags for storage in a cooler while transported to UW-Madison where they were immediately placed in cold storage at 4°C until processing. Within 48 h of collection, soils were sieved (2 mm) free of rocks and roots and hand homogenized. Subsamples (3 g) were weighed out, lyophilized, and stored at –20°C until used for lipid extraction analysis.

Membrane lipids were extracted from the soil (White and Ringelberg, 1998), purified, and identified using steps from a modified Bligh and Dyer (1959) method for phospholipid fatty acid extraction (PLFA), which was combined with fatty acid methyl ester (FAME) analysis as described by Microbial ID Inc. (Hayward, CA). Lipids were extracted using a chloroform-methanol extraction with a phosphate buffer. Then, following the procedure for FAME given by Microbial ID Inc., fatty acids were saponified by adding sodium hydroxide, followed by strong acid methanolysis. A 2- $\mu$ l

**Table 1**

Composition of guilds from chemically similar fatty acids used in the analysis. Only fatty acids that were identifiable and present in amounts greater than 0.5 mol% were used. Analysis was performed on measures of absolute abundance (nmol lipid g soil<sup>-1</sup>).

Guild	Generally indicates	Fatty acids included
Branched	Gram-positive bacteria <sup>a,b</sup> Actinomycetes <sup>c,d</sup>	a15:0, i15:0, a17:0, and i17:0 10Me16:0 and 10Me18:0
Monounsaturated	Gram-negative bacteria <sup>b,e</sup>	cis16:1 $\omega$ 7, cis18:1 $\omega$ 5, and cis18:1 $\omega$ 7
Cyclopropyl	Anaerobic bacteria <sup>e</sup> Mycorrhizal fungi (AMF) <sup>f</sup> Saprotrophic fungi (SF) <sup>c,f</sup>	cy17:0 and cy19:0 cis16:1 $\omega$ 5 cis18:1 $\omega$ 9 and cis18:2 $\omega$ 6,9

Referenced from: a. (Zelles et al., 1992); b. (Frostegård et al., 1993); c. (Federle et al., 1986); d. (Vestal and White, 1989); e. (Wilkinson, 1988); f. (Balsler et al., 2005; Olsson, 1999; Wilson et al., 2009).

FAME aliquot was analyzed using a Hewlett-Packard 6890 gas chromatograph equipped with a flame ionization detector and an Ultra 2 capillary column (Agilent Technologies, Santa Clara, CA). The gas chromatograph method and lipid peak identification were carried out using MIDI peak identification software and bacterial fatty acid standards (Sherlock Microbial Identification System, MIDI Inc., Newark, DE). Lipid biomarkers were quantified and converted to nmol lipid g soil<sup>-1</sup> using peak areas from two internal standards of known concentrations, nonanoic methyl ester (9:0) and nonadecanoic methyl ester (19:0).

Total nmol lipid g soil<sup>-1</sup> (absolute abundance of all identifiable lipids with C chains <20) was used as an index of microbial biomass, and abundance of signature lipids (Table 1) was used to quantify microbial groups representing segments of the microbial community, commonly referred to as 'guilds' (Balser et al., 2005; White et al., 1979; Zelles, 1999).

### 2.3. Statistical analysis

Before addressing the effects of cropping system treatments during our study period, we evaluated the effects of land use legacy (i.e., blocks 1–3 in corn and blocks 4 and 5 in grass-alfalfa) with PERMANOVA. Separating samples by past land use accounted for ~7% of the variability in the 2008 data ( $P=0.04$ ), but only 1.1% of variability in 2013 ( $P=0.64$ ), indicating these effects were relatively small at the onset of our study and had disappeared statistically 5 years later.

Further analyses were carried out in the R statistical environment (R Core Team, 2014). Total microbial lipids and marker lipids for individual functional groups were analyzed separately using linear mixed effects models with the *lmer* function in the 'lme4' package (Bates et al., 2014). The model was fully factorial, with each combination of *cropping system* and *stover removal/fertilization* represented as a level in a combined factor, while *block* and *plot within block* were included as random effects to reflect the split-plot design of the experiment as well as within-plot sampling in 2008. Because of the balanced experimental design, the model estimates equaled the arithmetic mean for each treatment combination. Orthogonal contrasts were made between each treatment and the 2008 baseline. In addition, all 2013 treatments

were compared to each other. Significance of individual contrasts were evaluated using false discovery rate corrections from the *lsmeans* function in the 'lsmeans' package (Lenth, 2013).

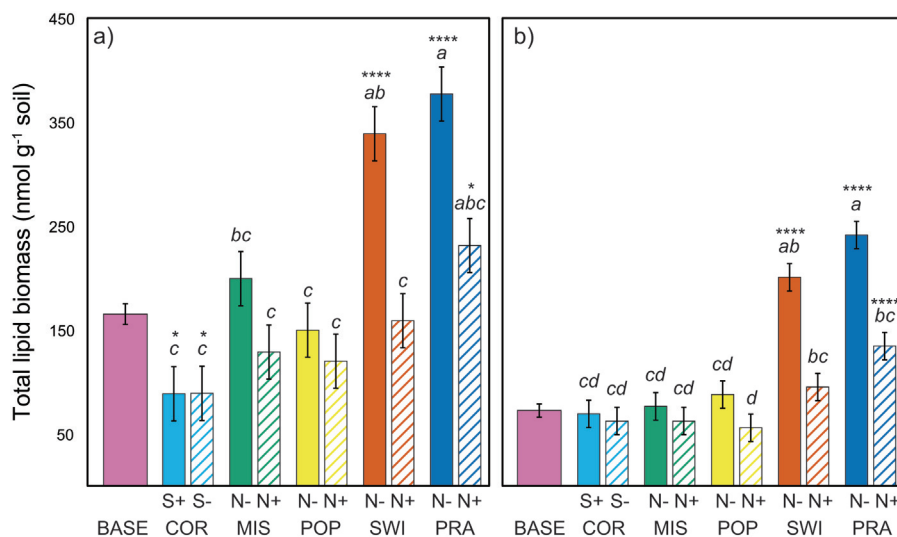
Multivariate statistics were conducted using the *adonis* (multivariate analysis of variance) and *metaMDS* (ordination) functions in the package 'vegan' (Oksanen et al., 2016). Ordination was conducted on arcsine-square root transformed lipid biomass values using Euclidian distances. The maximum number of random starts was increased to 200 to ensure a stable solution was always returned. For each ordination, *metaMDS* was called iteratively six times, each time using the previous best solution as the starting point.

## 3. Results

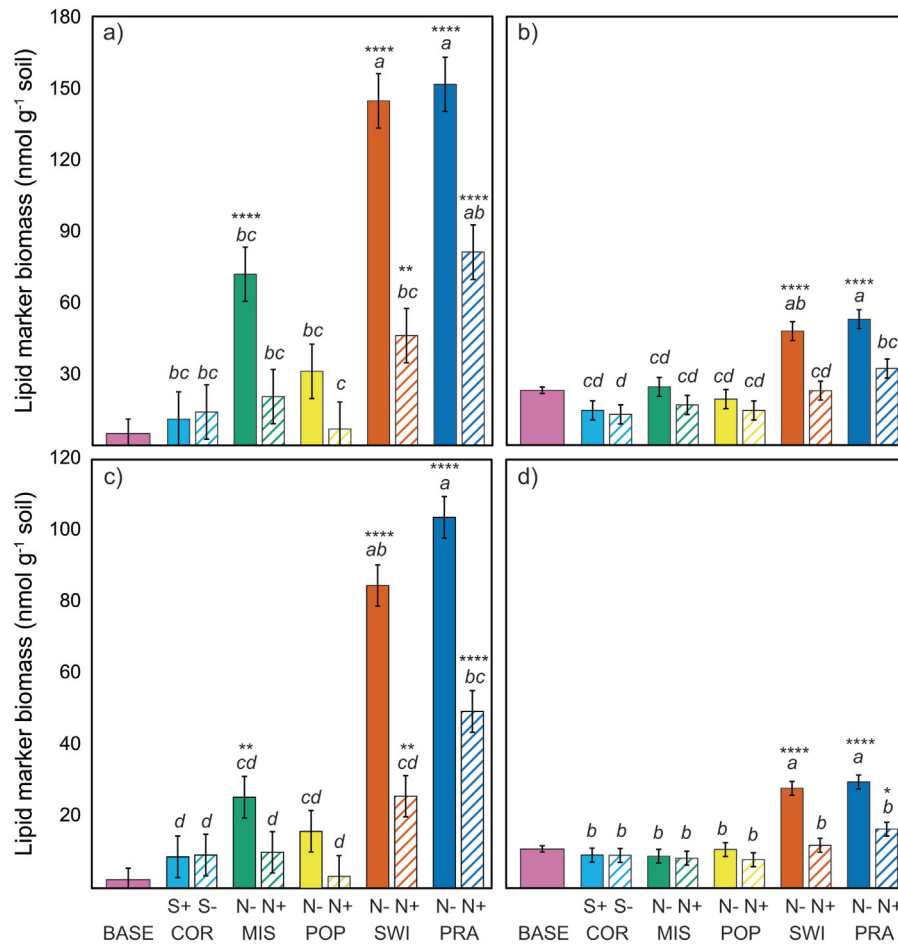
### 3.1. Cropping system effects on microbial biomass

Total microbial lipid biomass in unfertilized switchgrass and both fertilized and unfertilized prairie was significantly greater than the baseline values at both soil depths (Fig. 1). By contrast, total microbial lipid biomass in corn was reduced from baseline values at the surface (0–10 cm), but not the subsurface (10–25 cm). At both depths, microbial biomass for the corn, miscanthus, and poplar systems was lower than for unfertilized prairie and switchgrass. Fertilized plots corresponded to lower total microbial biomass in switchgrass at the surface and both switchgrass and prairie at the subsurface. Stover removal in corn had no effect on microbial biomass at either depth.

Arbuscular mycorrhizal fungi (AMF) and Gram-negative bacteria (Gm-) exhibited dynamics similar to total microbial biomass (Fig. 2). AMF biomass in both unfertilized and fertilized prairie and switchgrass, and unfertilized miscanthus increased significantly from baseline values (Fig. 2a, c), while N addition was linked to reduced AMF biomass in all three systems. Gm- biomass was less variable, and was only increased from the baseline in unfertilized switchgrass and prairie; in both systems Gm- biomass was greater without addition of N (Fig. 2b, d). AMF and Gm- dynamics were similar at both depths, although biomass values averaged 45% lower in the subsurface soils (10–25 cm). Biomass of Gram-positive bacteria (Gm+) did not vary among cropping systems or their



**Fig. 1.** Total microbial lipid biomass at (a) 0–10 cm, (b) 10–25 cm depth. Treatments are abbreviated as: BASE, 2008 baseline; COR, continuous corn; MIS, miscanthus monoculture; POP, poplar monoculture; SWI, switchgrass monoculture; PRA, prairie species assemblage. Bar shadings correspond to main or sub-plot treatments: S+, stover left on; S-, stover removed; N+, receiving N addition; N-, receiving no N addition. Error bars indicate  $\pm 1$  s.e. Within a panel, groups sharing a letter were not significantly different at  $P < 0.05$ . Significance of differences between treatments and the 2008 baseline are denoted by: \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ ; \*\*\*\*,  $P < 0.0001$ .



**Fig. 2.** Lipid biomarker biomass: (a) arbuscular mycorrhizal fungi 0–10 cm; (b) Gram-negative bacteria, 0–10 cm; (c) arbuscular mycorrhizal fungi 10–25 cm; (d) Gram-negative bacteria, 10–25 cm. Treatments are abbreviated as: BASE, 2008 baseline; COR, continuous corn; MIS, miscanthus monoculture; POP, poplar monoculture; SWI, switchgrass monoculture; PRA, prairie species assemblage. Bar shadings correspond to main or sub-plot treatments: S+, stover left on; S-, stover removed; N+, receiving N addition; N-, receiving no N addition. Error bars indicate  $\pm 1$  s.e. Within a panel, groups sharing a letter were not significantly different at  $P < 0.05$ . Significance of differences between treatments and the 2008 baseline are denoted by: \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ ; \*\*\*\*,  $P < 0.0001$ .

sub-plots. Gm+ biomass declined significantly from baseline values at the surface, but was equivalent to baseline values at the subsurface (not shown).

### 3.2. Cropping systems and N addition drive microbial composition

The influence of sampling year (i.e. distinction between baseline and post-establishment samples), cropping system, and management on microbial composition, as determined by permutational analysis of variance, was similar for both soil depths (Table 2). Baseline and post-establishment samples were clearly distinct. Among post-establishment samples, cropping system and N addition influenced composition patterns while stover retention and the interaction of N addition with cropping systems did not. NMDS effectively captured dissimilarities among samples at both depths with 2-dimensional ordinations (Fig. 3). These produced low stress values and strong correlations between observed dissimilarities and ordination distances (non-metric fit  $R^2 > 0.99$ ). Ordinations for both soil depths were highly correlated (Procrustes rotation  $R^2 = 0.85$ ,  $P < 0.0001$ ). Ordination largely recapitulated the variance structure described by permutational analysis of variance. Baseline samples formed a distinct cluster that was weakly associated with Gm+ biomass. Post-establishment samples separated by cropping system along a gradient of

**Table 2**

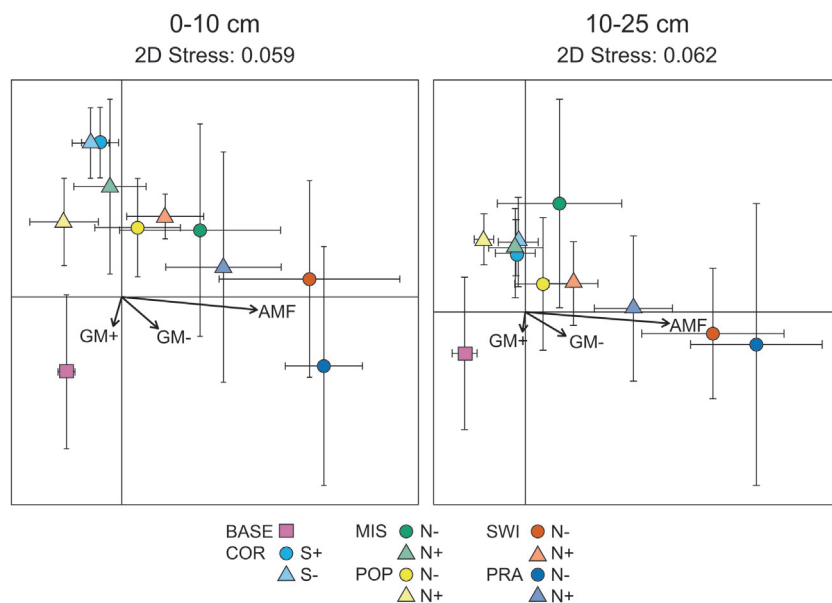
Permutational analysis of variance factors governing soil microbial community patterns.

Soil depth	Factor	SS	Pseudo-F	$R^2$
0–10 cm	Sampling year <sup>a</sup>	1.17	81.4 ***b	0.368
	Cropping system	0.60	10.4 ***	0.188
	N addition	0.10	7.2 ***	0.033
	Stover removal	0.01	0.5	0.002
	System $\times$ N addition	0.08	1.9	0.025
	Residual variance	1.25	–	0.385
10–25 cm	Sampling year <sup>a</sup>	1.02	54.4 ***	0.302
	Cropping system	0.53	7.1 ***	0.159
	N addition	0.12	6.2 ***	0.034
	Stover removal	0.01	0.4	0.002
	System $\times$ N addition	0.10	1.9	0.034
	Residual variance	1.59	–	0.472

\*\*\*, variance explained by factor significant at  $P < 0.001$  based on 9999 bootstrap permutations.

<sup>a</sup> Sampling year distinguishes between baseline and post-establishment systems.

increasing AMF and Gm- biomass, with corn, miscanthus, and poplar systems clustering closer to the baseline samples. N fertilization shifted samples toward the baseline, lower AMF and Gm- biomass end of the gradient.



**Fig. 3.** Nonmetric multidimensional scaling ordination of soil microbial communities at two soil depths. Cropping systems are distinguished by color and abbreviated as: BASE, 2008 baseline; COR, continuous corn; MIS, miscanthus monoculture; POP, poplar monoculture; SWI, switchgrass monoculture; PRA, prairie species assemblage. Sub-plots with stover retention (S+) or N addition (N+) are indicated by circles and darker shading, while sub-plots with stover removal (S-) or no N addition (N-) are indicated by triangles and lighter shading. Symbols are placed on mean loading scores for each group, with error bars indicating  $\pm 1$  s.e. Arrows are average loadings for PLFA markers for arbuscular mycorrhizal fungi (AMF), Gram-negative bacterial (Gm-), and Gram-positive bacteria (Gm+).

#### 4. Discussion

Biomass of AMF and Gm- bacteria increased substantially in cropping systems that incorporated native grasses and received no nitrogen fertilization. These changes were most pronounced in the top 10 cm of soil. We previously reported membrane lipid data from the top 15 cm of the unfertilized prairie system, measured over the 3 years prior to the present study (Herzberger et al., 2014). AMF biomass in 2013 slightly exceeded values observed from 2010 to 2012, while Gm- biomass more than doubled from our earlier measurements; the difference in sampling depth likely accounts for these deviations, as microbial biomass and activity are concentrated near the soil surface. The AMF and Gm- biomass values from the unfertilized switchgrass and prairie systems matched those from a broad sample of switchgrass and prairie fields in south central Wisconsin with a minimum age of 10 years (Liang et al., 2012). Trajectories of microbial community change following grassland establishment differ among studies, as do microbial community compositions of “established” grasslands and prairies. McKinley et al. (2005) found greater microbial biomass in a 25-year restoration than a 7-year one and, unlike our study, saw a decrease in the relative abundance of AMF biomass. Jangid et al. (2010) similarly found increased microbial biomass from a 6-year restoration to 25-year and remnant prairies, while reporting substantially higher fungal:bacterial biomass ratios in the 6- and 25-year restorations than the remnants. Looking at a much finer timescale, Allison et al. (2005) found AMF biomass plateaued at approximately 10 years, then decreased in relative abundance as bacterial biomass increased rapidly. A key distinction between these studies and ours is that they rely on space for time substitutions, which risk confounding effects from pre-existing differences (Pickett, 1989), while the treatments in our study were replicated within a single site. Overall, the literature indicates considerable variability in the microbial communities of grasslands and prairies, and suggests the communities in our system may continue to evolve over time.

Plant-associated soil microbes, including many AMF and Gm- bacteria, play a central role in the nutrient cycling and productivity of cropping systems (van der Heijden et al., 2008). The increase in AMF and Gm- biomass could signal a restoration of desirable microbially-mediated properties associated with natural ecosystems, such as more efficient nutrient cycling (Gliessman, 2007). One possible example of this was the absence of a yield difference between fertilized and unfertilized switchgrass and prairie (Duran et al., 2016; Sanford et al., 2016). Terrer et al. (2016) reported that microbial associations enable plants to overcome nitrogen limitations, and although they found no universal benefit of AMF association, Weremijewicz et al. (2016) show *Andropogon gerardii*, a C4 perennial grass present in our prairie system, can satisfy a substantial proportion of its nitrogen requirement through AMF association. Although most differences among treatments incorporating native grasses lacked statistical significance, the ranking of microbial biomass for these treatments matches the rankings of their N<sub>2</sub>O emissions and potential NO<sub>3</sub><sup>-</sup> leaching (Duran et al., 2016; Oates et al., 2016). While we cannot demonstrate causality in these relationships, microbial immobilization provides a major sink for soil nitrogen (Schimel and Bennett, 2004), potentially explaining this relationship.

Our findings illustrate the importance of considering the effects of N addition and native plant species when designing and establishing bioenergy cropping systems. While low baseline values of AMF may have been a result of tillage occurring at the site prior to cropping system establishment (van Groenigen et al., 2010), tillage cessation alone was probably not sufficient to increase microbial biomass to the levels we observed over 5 years (Helgason et al., 2010). Instead, symbiotic interactions with plants likely accelerated the development of AMF biomass (Allison et al., 2005). Nitrogen addition could disrupt this interaction by increasing nutrient availability to plants and thereby reducing their allocation of C to their associated microbiota (Johnson et al., 2010) and this mechanism may underpin the generalized negative relationship between N addition and the relative abundance of



AMF DNA in soil (Leff et al., 2015). The availability of plants suitable for host-specific interactions may have also played a role, as many AMF taxa have strong host preferences (Vandenkoornhuyse et al., 2003). The native species present in the switchgrass and prairie systems would thus form stronger interactions with the local AMF than introduced large woody plants such as poplars, which rarely associate with AMF, or exotic grasses that are unlikely to respond effectively to locally-adapted AMF taxa. In the absence of N addition, native species cropping systems in our study needed only 5 years to develop AMF biomass levels comparable to those found in long-term switchgrass stands and prairie restorations in south central Wisconsin (Liang et al., 2012), which likely facilitated increased plant N uptake.

Within a soil depth, Gm- biomass diverged from baseline values only in the unfertilized switchgrass and prairie systems. We hypothesize that much of the variation in Gm- biomass stemmed from fluctuations in the population of plant-associated diazotrophs (Liang et al., 2011). Both the switchgrass and prairie systems are theoretically capable of supporting biological N fixation, as the seeding mixture for the prairie contained native legumes (Herzberger et al., 2014) and switchgrass is capable of low levels of N fixation (Samson et al., 2005). However, the absence of a Gm- response to N addition in the miscanthus system, which also possesses N fixation capacity (Davis et al., 2009), somewhat undermines this hypothesis. Not all plant species show changes in their associated microbial communities in response to N limitation. For instance, urea-fertilized and unfertilized sorghum plants had virtually identical rhizosphere communities (Lavecchia et al., 2015). Corn varieties with higher N use efficiency cultivate larger, more active soil microbial communities (Pathan et al., 2015), demonstrating that even within a species there is variation in how N limitation alters plant-microbe interactions. The range of responses to N fertilization we observed among the cropping systems could thus indicate variability in the capacity to mobilize local microbial communities in response to N needs.

In contrast to both AMF and Gm- bacteria, Gm+ biomass did not vary across cropping systems or management, but differed systematically from the baseline in the surface soil (0–10 cm). The lack of response to cropping system and management strategy in the Gm+ bacteria is consistent with the abundance of free-living, resilient taxa within that guild (Paul and Clark, 1996). Post-establishment Gm+ biomass values were substantially lower than values reported from agricultural and perennial grass fields throughout south central Wisconsin (Liang et al., 2012), suggesting the process of preparing the study site reduced Gm+ biomass. Environmental stresses during establishment of the experimental site may have induced sporulation in much of the Gm+ population. These structures are more difficult to extract (Frostegård et al., 1999) and have a reduced surface area; both facts would reduce the prevalence of extractable lipids associated with Gm+ bacteria. Prior studies have indicated it takes about 10 years after prairie establishment for substantial increases in Gm+ biomass (Allison et al., 2005).

Soil microbial composition largely reflected the dynamics observed in the three guilds described above—baseline values were distinguished by Gm+ biomass while AMF and Gm- formed the gradient that separated the post-establishment systems. Community-level analysis illustrated that corn stover retention had little effect on the microbial community. This was unexpected, given how strongly plant litter and biomass inputs affect decomposition and soil respiration (Wardle et al., 1999; Zak et al., 2003). The absence of tillage may have slowed introduction of crop residues into the soil, which would reduce the effect of this management approach. Over longer time periods, stover removal should have larger impacts on soil C, and thus on the composition of the microbial community (Wilhelm et al., 2004). The similarity of

surface and subsurface soils at the community level demonstrated the influence of perennial grasses, notably switchgrass and other prairie species, deeper into the soil profile than the typically-studied “rooting zone” (Post and Kwon, 2000). Because microbial biomass is a key precursor to stable soil organic matter (Kallenbach et al., 2015), effect on the composition and stimulation of subsurface microbial biomass could result in substantial C storage deeper into the soil profile.

Low-input perennial monocultures and polycultures grown in an agronomic context have received little attention, so there is limited information on the potential for these novel cropping systems to alter soil microbial composition. Seasonal dynamics may greatly affect soil microbial communities (Contosta et al., 2015; Kramer et al., 2013), but in our randomized and replicated field experiment, a comparison of microbial communities under multiple cropping systems at the peak of the growing season provided meaningful insights into their ecological function.

## 5. Conclusions

We found N addition and cropping system strongly influenced soil microbial communities, particularly AMF and Gm- bacteria. Given plant-associated microbes play a significant role in ecosystem-level nutrient cycling, management may be a means of influencing the ecological properties of bioenergy cropping systems. Microbial composition emerging from unfertilized systems that incorporated native plant species resembled those found in undisturbed systems and long-term restorations, which could signal a reclamation of desirable microbially-mediated ecosystem properties. Our findings illustrate the importance of considering the effects of N addition and inclusion of native plant species when designing and establishing bioenergy cropping systems.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.agee.2016.09.008>.

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